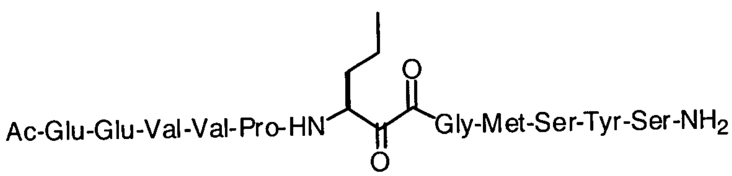
Dichloromethane was then added to the resin and the mixture was stirred for five minutes. This was followed by the addition of 20% acetic acid in water (4 mL). After stirring for 20 minutes, the resin was filtered using a fritted funnel and the dichloromethane was removed under reduced pressure. Hexane was added to the remaining residue and the mixture was agitated, and the layers separated (this was repeated twice to remove scavengers). Meanwhile, the resin was soaked in 1 mL methanol. The aqueous layer (20% HOAc) was added back to the resin and the mixture was agitated for five minutes and then filtered. The methanol was removed under reduced pressure and the aqueous layer was lyophilized. The peptide was then dissolved in 10-25% methanol (containing 0.1% trifluoroacetic acid) and purified by reverse phase HPLC.

Example I:

Synthesis of Ac-EEVVP-nV-(CO)-GMSYS-Am:

A2 cont'd.



Step I. Synthesis of Fmoc-Met-Ser(tBu)-Tyr(tBu)-Ser(tBu)-MBHA resin (SEQ ID NO: 53):

MBHA resin (10g, 4.6 mmol) was placed in a 250 mL fritted reaction vessel equipped with a nitrogen inlet. The resin was neutralized with 5% diisopropylethylamine in dimethylformamide (2 X 15 minutes). The resin was then washed twice with 15 mL dimethylformamide followed by three times with 15 mL portions of dichloromethane and dimethylformamide, respectively. a) Fmoc-

Please replace page 39 with: - -

A3 cont'd.

obtain a white solid (3.99 g, 94%). Thin layer chromatography in 9:1 dichloromethane: methanol showed two spots (syn and anti isomers) with R_f =

0.03 and 0.13, respectively. NMR δ ppm (CD_3OD): 7.75 (m, 2H), 7.6 (m, 3H), 7.2-7.4 (m, 14H), 6.1-6.2 (m, 1H), 4.25-4.4 (m, 2H), 4.1-4.2 (m, 2H), 3.85 (s, 2H), 1.6-1.8 (m, 2H), 1.3-1.5 (m, 2H), 0.95 (t, 3H).

Step III. Synthesis of Ac-Glu(OtBu)-Glu(OtBu)-Val-Val-Pro-OH (SEQ ID NO: 54):

a) Synthesis of Fmoc-Val-Pro-2ClTrt resin

In a 1L solid phase reaction vessel equipped with a nitrogen inlet, 25 g of Pro-2ClTrt resin (200-400 mesh, 0.64 mmol/g substitution) was suspended in dimethylformamide (213 mL). Fmoc-Val-OH (1.5 g, 32 mmol) was coupled for four hours according to Procedure A. A small aliquot was taken for colorimetric ninhydrin analysis which showed a 99.5% coupling efficiency in the production of the title compound.

b) Synthesis of Fmoc-Val-Val-Pro-2ClTrt resin

The resin from the previous step (0.53 mmol/g) was deprotected according to Procedure B. It was then coupled to Fmoc-Val-OH (10.85 g, 32 mmol) according to Procedure A with 99.5% efficiency.

A3 cont'd.

c) Synthesis of Fmoc-Glu(OtBu)-Val-Val-Pro-2ClTrt resin (SEQ ID NO: 55)

The resin from the previous step (0.504 mmol/g) was deprotected according to Procedure B. It was then coupled to Fmoc-Glu(OtBu)-OH (13.63 g, 32 mmol) according to Procedure A with 99.4% efficiency.

d) Synthesis of Fmoc-Glu(OtBu)-Glu(OtBu)-Val-Val-Pro-2ClTrt resin (SEQ ID NO: 56)

The resin from the previous step (0.461 mmol/g) was deprotected according to Procedure B. It was then coupled to Fmoc-Glu(OtBu)-OH (13.63 g, 32 mmol) according to procedure A with 99.2% efficiency to yield the titled compound.

e) Synthesis of Ac-Glu(OtBu)-Glu(OtBu)-Val-Val-Pro-2ClTrt resin (SEQ ID NO: 57)

The resin from the previous step (0.42 mmol/g) was deprotected according to procedure B. The N-terminus was then capped according to Procedure C to yield the desired compound in 99.7% efficiency.

Please replace page 40 with: - -

f) Synthesis of Ac-Glu(OtBu)-Glu(OtBu)-Val-Val-Pro-OH (SEQ ID NO: 58)

The resin from the previous step was transferred to a 1L plastic bottle and cleaved in the presence of 525 ml solution of acetic acid: trifluoroethanol: dichloromethane (1:1:3) with vigorous shaking for two hours. The resin was filtered using a fritted funnel and washed 3 × 50 mL with dichloromethane. The brownish red filtrate was concentrated to an oil which was then treated three times with 50 ml of a 1:1 mixture of dichloromethane: n-heptane. The crude off-white powder (13 g) was then dissolved in minimum amount of methanol and purified by HPLC using a 2.2 X 25 cm reverse phase column, containing a C-18 resin comprised of 10 micron size gel particles with a 300 angstrom pore size, eluting with a gradient ranging from 15-55% acetonitrile in water. The pure fractions were pulled and concentrated to a fluffy, white product (7.5 g, 65%). Analytical HPLC using a 4.6 X 250 mm reverse phase column, containing a C-18 resin comprised of 5 micron size gel particles with a 300 angstrom pore size ran at 5-50% acetonitrile (containing 0.1% trifluoroacetic acid) showed one peak with the retention time of 20.5 min. Low resolution mass spectrum confirmed the desired mass (MH^+ 726.5).

A4 cont'd.

Step IV. Synthesis of Fmoc-nVal(dpsc)-Gly-Met-Ser(tBu)-Tyr(tBu)-Ser(tBu)-MBHA (SEQ ID NO: 59):

The resin obtained from step I (2 g, 0.66 mmol) was deprotected according to Procedure B. Fmoc-nVal(dpsc)-Gly-OH (step IIf) (1.1 g, 1.7 mmol) was then coupled over 18 hours according to procedure A using N-methylpyrrolidine as solvent with 98% efficiency (2 g resin obtained, new resin substitution determined to be 0.276 mmol/g).

Step V. Synthesis of Ac-Glu(OtBu)-Glu(OtBu)-Val-Val-Pro-nVal(dpsc)-Gly-Met-Ser(tBu)-Tyr(tBu)-Ser(tBu)-MBHA (SEQ ID NO: 60):

1 g resin (0.28mmol) from step IV was placed in a fritted reaction vessel. The resin was deprotected according to Procedure B. Ac-Glu(OtBu)-Glu(OtBu)-

a4 concl'd

Val-Val-Pro-OH (400 mg, 0.55 mmol) (obtained in IIIf) was then coupled over 18 hours according to Procedure A with 98% efficiency (978 mg resin obtained).

Please replace page 41 with: - -

Step VI. Synthesis of Ac-Glu-Glu-Val-Val-Pro-nVal(CO)-Gly-Met-Ser-Tyr-Ser-MBHA (SEQ ID NO: 61):

The resin from step V (998 mg) was treated for one hour with 10 ml dichloromethane: trifluoroacetic acid (1:1). The reactants were drained and the resin was thoroughly washed with dichloromethane. The resin was subjected to semicarbazone deprotection Procedure D and dried under vacuum to yield 943 mg resin.

a5 cont'd.

Step VII. Synthesis of Ac-Glu-Glu-Val-Val-Pro-nVal(CO)-Gly-Met-Ser-Tyr-Ser-NH2 (SEQ ID NO: 62):

The resin obtained from step VI (942.8 mg) was cleaved with HF according to Procedure E. The crude product (314 mg) was subjected to HPLC purification using a 2.2 X 25 cm reverse phase column, containing a C-18 resin comprised of 10 micron size gel particles with a 300 angstrom pore size, eluting with a gradient using 0-30% (30 minutes) acetonitrile in water followed by 30-75% (10 minutes) acetonitrile in water. The desired fractions were pulled and concentrated to a white solid (238 mg, 26%). Analytical HPLC using a 4.6 X 250 mm reverse phase column, containing a C-18 resin comprised of 5 micron size gel particles with a 300 angstrom pore size, eluting at 5-50% acetonitrile (containing 0.1% trifluoroacetic acid) showed one peak at 13 minutes. Low resolution mass spectrum confirmed the desired mass (MH^+ 1265.6).The Table below lists the synthesis of other similar compounds:

Table of 11mer Compounds Synthesized according to Example 1

COMPOUND NAME	SYNTHESIS

a5 cond'd.

AcEEVVPnV-(CO)-GMSYS-Am (SEQ ID NO: 5)	example I
AcEEVVPnV-CO-GMdSYS-Am (SEQ ID NO: 6)	step Ic: used Fmoc-dSer(tBu)-OH
AcEEVVPnV-CO-GMdHYS-Am (SEQ ID NO: 7)	step Ic: used Fmoc-dHis(Trt)-OH
AcEEVVPnV-CO-GMdDYS-Am (SEQ ID NO: 8)	step Ic: used Fmoc-dAsp(tBu)-OH
AcEEVVPnV-CO-GdMSYS-Am (SEQ ID NO: 9)	step Id: used Fmoc-dMet-OH

Please replace page 42 with : - -

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a6 cont'd.

AcEEVVPnV-CO-GdMdSYS-Am (SEQ ID NO: 10)	step Ic: used Fmoc-Ser(tBu)-OH, step Id: used Fmoc-dMet-OH
AcEEVVPnV-CO-GdMHYS-Am (SEQ ID NO: 11)	step Ic: used Fmoc-His(Trt)-OH, step Id: used Fmoc-dMet-OH
AcEEVVPnV-CO-GdMDYS-Am (SEQ ID NO: 12)	step Ic: used Fmoc-Asp(OtBu)-OH, step Id: used Fmoc-dMet-OH
AcEEVVPnV-CO-GdMdDYS-Am (SEQ ID NO: 13)	step Ic: used Fmoc-dAsp(OtBu)-OH, step 1d: used Fmoc-dMet-OH
AcEEVVPnV-CO-GGSYS-Am (SEQ ID NO: 14)	step Id: used Fmoc-Gly-OH
AcEEVVPnV-CO-GGHYS-Am (SEQ ID NO: 15)	step Ic: used Fmoc-His(Trt)-OH, step Id: used Fmoc-Gly-OH
AcEEVVPnV-CO-GGdHYS-Am (SEQ ID NO: 16)	step Ic: used Fmoc-dHis(Trt)-OH, step Id: used Fmoc-Gly-OH
AcEEVVPnV-CO-GGDYS-Am (SEQ ID NO: 17)	step Ic: used Fmoc-Asp(OtBu)-OH, step Id: used Fmoc-Gly-OH

AcEEVVPnV-CO-GGdDYS-Am (SEQ ID NO: 18)	step Ic: used Fmoc-dAsp(OtBu)-OH, step Id: used Fmoc-Gly-OH
AcEEVVPnV-CO-GQSYS-Am (SEQ ID NO: 19)	step Id: used Fmoc-Gln(Trt)-OH
AcEEVVPnV-CO-GQdSYS-Am (SEQ ID NO: 20)	step Ic: used Fmoc-dSer(tBu)-OH, step Id: used Fmoc-Gln(Trt)-OH
AcEEVVPnV-CO-GQdHYS-Am (SEQ ID NO: 21)	step Ic: used Fmoc-dHis(Trt)-OH, step Id: used Fmoc-Gln(Trt)-OH
AcEEVVPnV-CO-GQdDYS-Am (SEQ ID NO: 22)	step Ic: used Fmoc-dAsp(OtBu)-OH, step Id: used Fmoc-Gln(Trt)-OH
AcEEVVPnV-CO-GdQSYS-Am (SEQ ID NO: 23)	step Id: used Fmoc-dGln(Trt)-OH
AcEEVVPnV-CO-GdQdSYS-Am (SEQ ID NO: 24)	step Ic: used Fmoc-dSer(tBu)-OH, step Id: used Fmoc-dGln(Trt)-OH
AcEEVVPnV-CO-GdQHYS-Am (SEQ ID NO: 25)	step Ic: used Fmoc-His(Trt)-OH, step Id: used Fmoc-dGln(Trt)-OH

A6 cont'd.

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Please replace page 43 with: - -

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AcEEVVPnV-CO-GdQDYS-Am (SEQ ID NO: 26)	step Ic: used Fmoc-Asp(OtBu)-OH, step Id: used Fmoc-dGln(Trt)-OH
AcEEVVPnV-CO-GdQdDYS-Am (SEQ ID NO: 27)	step 1c: used Fmoc-dAsp(OtBu)-OH, step Id: used Fmoc-dGln(Trt)-OH
AcEEVVPnV-CO-GTSYS-Am (SEQ ID NO: 28)	step Id: used Fmoc-Thr(tBu)-OH
AcEEVVPnV-CO-GTdSYS-Am (SEQ ID NO: 29)	step Ic: used Fmoc-dSer(tBu)-OH, step Id: used Fmoc-Thr(tBu)-OH
AcEEVVPnV-CO-GTHYS-Am (SEQ ID NO: 30)	step Ic: used Fmoc-His(Trt)-OH, step Id: used Fmoc-Thr-OH

A7 cont'd.

AcEEVVPnV-CO-GTDYS-Am (SEQ ID NO: 31)	step Ic: used Fmoc-Asp(OtBu)0H, step Id: use Fmoc-Thr(tBu)-OH
AcEEVVPnV-CO-GTdDYS-Am (SEQ ID NO: 32)	step Ic: used Fmoc-dAsp(OtBu)-OH, step Id: used Fmoc-Thr(tBu)-OH
AcEEVVPnV-CO-GSdSYS-Am (SEQ ID NO: 33)	step Ic: used Fmoc-dSer(tBu)-OH, step Id: used Fmoc-Ser(tBu)-OH
AcEEVVPnV-CO-GSdHYS-Am (SEQ ID NO: 34)	step Ic: used Fmoc-dHis(Trt)-OH, step Id: used Fmoc-Ser(tBu)-OH
AcEEVVPnV-CO-GSdDYS-Am (SEQ ID NO: 35)	step Ic: used Fmoc-dAsp(OtBu)-OH, step Id: used Fmoc-Ser(tBu)-OH
AcEEVVPnV-CO-GdSSYS-Am (SEQ ID NO: 36)	step Id: used Fmoc-dSer(tBu)-OH
AcEEVVPnV-CO-GdSdSYS-Am (SEQ ID NO: 37)	step Ic: used Fmoc-dSer(tBu)-OH, step Id: used Fmoc-d-Ser(tBu)-OH
AcEEVVPnV-CO-GdSHYS-Am (SEQ ID NO: 38)	step Ic: used Fmoc-His(Trt)-OH, step Id: used Fmoc-dSer(tBu)-OH
AcEEVVPnV-CO-GdSdHYS-Am (SEQ ID NO: 39)	step Ic: used Fmoc-dHis(Trt)-OH, step Id: used Fmoc-dSer(tBu)-OH
AcEEVVPnV-CO-GdSDYS-Am (SEQ ID NO: 40)	step Ic: used Fmoc-Asp(OtBu)-OH, step Id: used Fmoc-dSer(tBu)-OH
AcEEVVPnV-CO-GdSdDYS-Am (SEQ ID NO: 41)	step Ic: used Fmoc-dAsp(OtBu)-OH, step Id: used Fmoc-dSer(tBu)-OH

A7 concl'd.

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Please replace page 44 with: - -

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A8 cont'd.

AcEEVVPnV-CO-GM(O)HYS-Am (SEQ ID NO: 42)	step Ic: used Fmoc-His(Trt)-OH
AcEEVVPnV-(CO)-GdM(O)SYS-Am (SEQ ID NO: 43)	step Id: used Fmoc-dMet-OH

AcEEVVPnV-CO-GdM(O)dHYS-Am (SEQ ID NO: 44)	step Ic: used Fmoc-dHis(Trt)-OH, step Id: used Fmoc-dMet-OH
AcEEVVPnV-CO-GdM(O)DYS-Am (SEQ ID NO: 45)	step Ic: used Fmoc-Asp(OtBu)-OH, step Id: used Fmoc-dMet-OH
AcEEVVPnV-CO-GdM(O)dDYS-Am (SEQ ID NO: 46)	step Ic: used Fmoc-dAsp(OtBu)-OH, step Id: used Fmoc-dMet-OH
Ac-EEVVP-V-(CO)-GMSYS-Am (SEQ ID NO: 47)	step II (b1): used Fmoc-Val-OH
Ac-EEVVP-L-(CO)-GMSYS-Am (SEQ ID NO: 48)	step II (b1): used Fmoc-Leu-OH
Ac-EEVVP-nL-(CO)-GMSYS-Am (SEQ ID NO: 49)	step II (b1): used Fmoc-nLeu-OH
Ac-EEVVP-Abu-(CO)-GMSYS-Am (SEQ ID NO: 50)	step II (b1): used Fmoc-Abu-OH
Ac-EEVVP-(s,s)alloT-(CO)-GMSYS-Am (SEQ ID NO: 51)	step II (b1): used Fmoc-(s,s)alloThr-OH
Ac-EEVVP-G(propynyl)-(CO)-GMSYS-Am (SEQ ID NO: 52)	step II b1 used Fmoc-G(propynyl)-OH

A8 cont'd.

Assay for HCV Protease Inhibitory Activity:

Spectrophotometric Assay: Spectrophotometric assay for the HCV serine protease was performed on the inventive compounds by following the procedure described by R. Zhang *et al*, *Analytical Biochemistry*, 270 (1999) 268-275, the disclosure of which is incorporated herein by reference. The assay based on the proteolysis of chromogenic ester substrates is suitable for the continuous monitoring of HCV NS3 protease activity. The substrates were derived from the P side of the NS5A-NS5B junction sequence (Ac-DTEDVVX(Nva) (SEQ ID NO: 1), where X = A or P) whose C-terminal carboxyl groups were esterified with one of four different chromophoric alcohols (3- or 4-nitrophenol, 7-hydroxy-4-methyl-coumarin, or 4-phenylazophenol). Presented below are the synthesis, characterization and

Please replace page 47 with:-

pH 6.5, 300 mM NaCl, 10% glycerol, 0.05% lauryl maltoside, 5 μM EDTA and 5 μM DTT) were optimized for the NS3/NS4A heterodimer (D. L. Sali *et al, ibid.*)). Typically, 150 μl mixtures of buffer, substrate and inhibitor were placed in wells (final concentration of DMSO 4 % v/v) and allowed to preincubate at 30 °C for approximately 3 minutes. Fifty μls of prewarmed protease (12 nM, 30°C) in assay buffer, was then used to initiate the reaction (final volume 200 μl).The plates were monitored over the length of the assay (60 minutes) for change in absorbance at the appropriate wavelength (340 nm for 3-Np and HMC, 370 nm for PAP, and 400 nm for 4-Np) using a Spectromax Plus microtiter plate reader equipped with a monochrometer (acceptable results can be obtained with plate readers that utilize cutoff filters). Proteolytic cleavage of the ester linkage between the Nva and the chromophore was monitored at the appropriate wavelength against a no enzyme blank as a control for non-enzymatic hydrolysis. The evaluation of substrate kinetic parameters was performed over a 30-fold substrate concentration range (~6-200 μM). Initial velocities were determined using linear regression and kinetic constants were obtained by fitting the data to the Michaelis-Menten equation using non-linear regression analysis (Mac Curve Fit 1.1, K. Raner). Turnover numbers (k_{cat}) were calculated assuming the enzyme was fully active.

a9 cont'd.

Evaluation of Inhibitors and Inactivators: The inhibition constants (K_i) for the competitive inhibitors Ac-D-(D-Gla)-L-I-(Cha)-C-OH (27) (SEQ ID NO: 2), Ac-DTEDVVA(Nva)-OH (SEQ ID NO: 3) and Ac-DTEDVVP(Nva)-OH (SEQ ID NO: 4) were determined experimentally at fixed concentrations of enzyme and substrate by plotting v_o/v_i vs. inhibitor concentration ($[I]_o$) according to the rearranged Michaelis-Menten equation for competitive inhibition kinetics: $v_o/v_i = 1 + [I]_o/(K_i(1 + [S]_o/K_m))$, where v_o is the uninhibited initial velocity, v_i is the initial velocity in the presence of inhibitor at any given inhibitor concentration ($[I]_o$) and $[S]_o$ is the substrate concentration used. The resulting data were fitted using

linear regression and the resulting slope, $1/(K_i(1+[S]_o/K_m)$, was used to calculate the K_i^* value.

a9 cont'd.

The obtained K_i^* values for the various compounds of the present invention are given in the afore-mentioned **Table** wherein the compounds have been - -

Remarks

Entry of the foregoing Amendment is respectfully requested. As of entry of the Amendment, claims 1-30 will be pending. The Amendment adds sequence identifiers after each peptide sequence appearing in the specification and in the Sequence Listing. The Amendment does not add any new matter to the Application.

Statement Under Rule 821

The content of the attached paper entitled "SEQUENCE LISTING" and of the accompanying identically labeled diskette, specifically the text file therein labeled "seqlist.txt", is the same. Furthermore, the information contained in the attached "SEQUENCE LISTING" and the text file is identical to the information originally submitted with the specification. Accordingly, no new matter has been added to the Application.

Respectfully submitted,

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